AMENDMENTS

The Examiner is respectfully requested to make the following amendments.

IN THE SPECIFICATION

On page 1, line 5:

This application is a divisional of U.S. Serial No. 09/989,259, filed November 20, 2001, now U.S. Patent No. 6,656,430, issued December 2, 2003, which is a divisional of U.S. Serial No. 09/614,834, filed July 20, 2000, now U.S. Patent No. 6,319,468, issued November 20, 2001, which is a divisional application of U.S. Serial No. 08/995,056, filed December 19, 1997, now U.S. Patent No. 6,143,247, issued November 7, 2000, which claims priority to U.S. Provisional Application Serial No. 60/034,327, filed December 20, 1996.

On page 12, line 7:

Figure 1 is a schematic illustration of affinity-based platforms of the invention. Figure 1A is comprised of a substrate **10** coated with a specific binding reagent comprising a first member of an affinity binding pair **11**. Figure 1B ~~illustrates~~ illustrates a substrate **10** coated with a reflective material **12**, which is coated with a specific binding reagent comprising a first member of an affinity binding pair **11**. Figure 1C is a substrate **10**, where the surface of the substrate is partially coated with a specific binding reagent comprising a first member of an affinity binding pair **11**, and with a patterned reflective material **12** which is in turn derivatized with a blocking agent **13**. Figure 1D is a substrate **14**, into which has been formed pits carrying encoded information. The lower surface of **14** is coated with a reflective coating **15**, and a protective layer **16**. The upper surface of **14** is coated with a specific binding reagent comprising a first member of an affinity binding pair **11**.

On page 12, lines 15-20:

~~Figure 2 is~~ Figures 2 and 2A set forth an exemplar arrangement of platform components useful for enumerating particulates in a fluid, for example cell counting, and comprises a sample input port **21** connected to an overflow chamber **23** *via* a fluid capillary **22**. The sample inlet port **21** is fluidically connected to the binding chamber **24**, which is in turn connected to a waste chamber

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25. Air displacement channels **26** facilitate filling of chambers. Wash buffer chambers **27** and **29**, and a dye chamber **28** are fluidically connected to the binding chamber **24** *via* fluid capillaries **22**.

On page 13, line 17:

Figure 4 contains illustrations of arrangements of platform components useful for studying the effect of a test molecule or molecules on populations of cells for enumerating particulates in a fluid, for example cell counting. Figure 4A comprises a test molecule chamber **40**, linked by a capillary **32** containing a valve **33** to a cell accumulation chamber **43**. The binding chamber is linked to a waste chamber **35**. Figure 4B incorporates a dye chamber **36** fluidically connected *via* a capillary **32** and valve **33** to the binding chamber **34**. Figure 4C provides an alternate arrangement of the components of Figure 4B. Figure 4D is similar to Figure 4C, but with the incorporation of a wash buffer chamber **31** in the fluidic path between the test molecule chamber **40** and the cell accumulation chamber **43**. Figures 4E and 4F are ~~Figure 4E is~~ similar to Figure 4A, with the exception of a multiplicity of test molecule chambers **40** arrayed serially (4E) or in parallel (4F). Figure 4~~F~~G is an arrangement in which fluid from a test molecule chamber **40** and a dilution buffer reservoir can be directed to receiving chambers **42** to provide serial dilutions of the test molecule solution. Figure 4H is another advantageous arrangement of the components of Figure 4G.

On page 14, line 15:

Figure 6 illustrates means and methods for counting and studying individual cells using platforms of the type Figure 1C. Figure 6A illustrates an optical apparatus or "head" derived from optical memory storage and retrieval devices for interrogating a platform **60**. Optical components include diode laser **65**, diffraction grating **64**, beam splitter **63**, collimating lenses **62**, focusing lens and actuator **61**, lens **66**, side lobe detectors **67**, and central detector **68**. Focusing and tracking are achieved through servo control circuits **69**, and actuators **61** and **71**. The signal derived from interaction of the central beam with the cell **74** (*e.g.* fluorescence emission) is detected by optical

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detector **68** and amplified by circuitry **70**. Figures 6B and 6C illustrate advantageous arrangements of reflective tracking features on the platform. In Figure 6B track widths **72** are modulated such that reflections from the two side lobes **73** produce a modulated signal. Amplitude or frequency modulation, achieved by varying the geometry of the tracks **75**, can be used to encode positional information. Figure 6C illustrates another arrangement where information is encoded by the presence or absence of reflective features in the central track.

On page 22, lines 6-8:

The apparatus of the invention also provides detection systems for detecting, monitoring, quantitating or analyzing particulates specifically retained on the surface of the platform, in a detection chamber comprising a specific binding reagent or in a cell accumulation chamber as described herein. Detection systems useful in the manufacture and use of the platforms of the invention include, but are not limited to, fluorescent, chemiluminescent, colorimetric, or scattering measurements. Figure 5 illustrates optical systems for effecting these measurements. The apparatus of Figure 5A, suitable for transmission, light scattering or direct fluorescence measurement of a platform such as illustrated in Figure 1A, comprises a light source **54**, focusing lens system **53**, assembly **51** comprising optical elements to collect, filter and focus light onto the photodetector **50**. Figure 5B incorporates the detection elements of Figure 5A, and would be suitable for chemiluminescence or bioluminescence measurements. Figure 5C is a rearrangement of the components of Figure 5A for use where the light is reflected from platforms of the type shown in Figure 1B. Figure 5D is an apparatus suitable for fluorescence detection on platforms shown in Figures 1A and 1C, where the assembly of optical elements ~~55~~ **55** includes elements such as excitation and emission filters, a dichroic mirror and lenses. Figure 5E is an apparatus suited for use with platforms shown in Figure 1D. The elements **56** comprise those necessary to read data from an optical disc such as a CD-ROM. electrochemical and radioactivity detecting means. Optionally, the detection system can be integral to the platform and can comprise a simple visual detection means such as the development of a visible color. Alternatively, the detection system can comprise a component of a device manipulating the platform, preferably comprising an optical detecting

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means. Also included in the invention are devices comprising a light source for illuminating the platform and a magnifying means to facilitate visual inspection (direct or computer-aided imaging) of the platform. Non-optical detection systems such as electrochemical and radioactivity detecting means may also be used. Embodiments wherein components of the detecting means comprise both the platform and the device are also encompassed by the invention.

On page 26, line 23:

The surface of the platform, particularly the area defining the detection or cell accumulation chamber, is also advantageously treated with a non-specific blocking agent or agents to prevent non-specific binding of particulates, particularly cells, to the surface of the platform. The nature and extent to which such treatments are necessary depends strongly on the nature of the surface. For example, a strong correlation has been established between water contact angle and cell adsorption, with hydrophilic surfaces showing significantly less cell adsorption than hydrophobic surfaces (*see* Ikada, 1994, *Biomaterials* 15: 725). Silicon, silica, and quartz present an inherently high-energy, hydrophilic surface. Alteration of surface properties is attained through hydroxylation (achieved, *for example*, by NaOH treatment at high temperatures) or silanization. Substituted silanes and siloxanes are particularly appropriate for increasing the hydrophilicity of an otherwise hydrophobic surface. These compounds consist of one or several reactive head-groups which bond (chemically or through hydrogen-bonding) to a substrate, for example, a core region of alkane ($-CH_2O-$). These compounds also provide a route for more sophisticated alteration of surface properties (such as derivation with functional groups to obtain the surface properties of interest). A wide variety of such functionalities can be introduced at a surface, including vinyl, phenyl, methylene and methoxy groups, as well as surfaces providing mixed functionalities. These functional groups not only change gross properties like liquid contact angle, but provide sites for preferential adsorption of molecules, either *per se* or as a result of further conjugation of specific binding reagents such as peptide ligands, antibodies and the like. More preferably, the surface is treated after deposition of the specific binding ~~regent(s)~~ reagent(s) with a non-specific blocking agent, including but not limited to bovine serum albumin and casein.

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, please replace "regents" with –reagents–.

On page 30, line 12:

The components of the platforms of the invention are in fluidic contract with one another. In preferred embodiments, fluidic contact is provided by microchannels comprising the surface of the platforms of the invention. Microchannel sizes are optimally determined by specific applications and by the amount of delivery rates required for each particular embodiment of the platforms and methods of the invention. Microchannel sizes can range from ~~0.1 m~~ 0.1μm to a value close to the 1mm thickness of the platform. Microchannel shapes can be trapezoid, circular or other geometric shapes as required. Microchannels preferably are embedded in a platform having a thickness of about 0.1 to 100mm, wherein the cross-sectional dimension of the microchannels across the thickness dimension of the platform is less than 500μm and from 1 to 90 percent of said cross-sectional dimension of the platform.

On page 32, line 19:

The orientation of these components of the detecting means of the apparatus of the invention will be understood to depend on the nature of the detection or cell accumulation chamber and the construction thereof. For example, platforms wherein the detection or cell accumulation chamber comprises an optically transparent surface (completely or in part, see ~~Figures 1A, 1C~~ Figures 1A and 1C) will advantageously be used with a device having the light source positioned on one side of the platform and the photodetector positioned on the other side of the platform. In alternative arrangements, the photodetector is arranged directly across from the light source (i.e. at an angle of about 180 degrees, as in Figure 5A) or more advantageously obliquely across the platform from the light source (i.e. at an angle of between 90 and 180 degrees). In the latter embodiments, the apparatus may also advantageously include a mirror or other means for deflecting the transmitted light to the photodetecting means; however, it will be recognized that such mirrors are not required in embodiments provided for fluorescence detection.

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On page 33, lines 2 and 5:

In alternative arrangements, wherein the surface of the platform at the detection or cell accumulation chamber comprises a reflecting surface (~~Figure 1B, 1D~~ Figures 1B and 1D), the photodetector is advantageously positioned on the same side of the platform as the light source (Figure 5C). In preferred embodiments, the photodetector is provided as an integrated component of an assembly comprising the light source (~~Figure 5D, 5E~~ Figures 5D and 5E), wherein the reflected light is detected along the same axis as the incident light (i.e. at about 0 degrees).

On page 34, line :

Detecting means derived from conventional CD or CD-ROM systems are also advantageously provided. The apparatus **56** in Figure 5E represents an optical "head" of a CD (see E.W. Williams, 1994, The CD-ROM and Optical Disc Recording Systems, Oxford University Press, New York) and is used with the platform of Figure 1D, the substrate of which contains data encoded in an industry standard format, in the form of pits stamped into the plastic matrix and coated with a reflective coating (*e.g.*, aluminum). The platform is constructed so that the aluminized pits are the focal point of the CD laser. Platform fluidics handling components are built within and upon this plastic layer. Particulates bound through the first member of the binding pair **11** to the surface of the thinner substrate **14** scatter light and interfere with the reading of the data. Those skilled in the art recognize that the thickness and index of refraction of optical storage media (e.g. CD-ROM) are such that the effect of light scattering by particles on the surface of the disk is reduced. In this application, the thickness and composition of the substrate, fluid layer and cover are chosen such that particulates bound to the surface interfere with the integrity of the data, and the errors generated in reading the data provide a measure of the number of particulates bound. The optical detection apparatus pictured in Figure 5D and in more detail in Figure 6A, is to be used with the platform of Figure 1C which contains reflective features such as those pictured in Figure 6B and 6C. Optical elements similar to those used in a conventional CD head are used to generate and focus a main beam and side beams on the surface of the platform. The reflection of the side beams off of the

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reflective features are used to track the central beam along the transparent regions of the platform, so that particulates bound there may be detected. Those skilled in the art recognize that optical data retrieval systems use the reflection of the central beam in a servo-controlled focusing system. This approach differs in that control of the focusing actuator is based on intensities of the reflections from the side beams off of the reflective features. The interaction of a particle with the central beam may result in fluorescence, absorption, or scattering, which may be detected by the detector ~~50~~ **50** within the "head" or by another detector advantageously placed (not shown). These embodiments thus provide cell sorting capability, cell tracking and cell viability information, whereby the status of the cells at each point can be detected and distinguished from each other cell. This capacity enables the platforms and devices of the invention to provide cell-specific data and tracking information.

On page 40, line 9:

Mixing elements, are also advantageously provided as components of the platforms of the invention. Static mixers can be incorporated into fluid handling structures of the platform by applying a textured surface to channels or chambers composing the mixer. Two or more channels can be joined at a position on the platform and their components mixed together by hydrodynamic activity imparted upon them by the textured surface of the mixing channel or chamber and, for example, by the action of centripetal force imparted by a rotating platform. Fluids can be mixed for the purposes of preparing serial dilutions of test compounds for subsequent transfer to the cell accumulation chamber, as is illustrated schematically in ~~Figure~~ Figures 4G and 4H. Mixing can also be accomplished by rapidly changing the direction of rotation and by physically agitating the platform by systems external thereto.

On page 51, lines 18-29:

An example of an assay for a mammalian cell in a biological fluid is the detection of somatic cell in a sample of cow's milk. The assay system is illustrated in ~~FIGURE 2~~ Figures 2 and 2A and consists of a sample entry port **21**, wash buffer chambers **27** and **29** (containing a wash buffer

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solution of 25 mM potassium hydrogen (KH) phthalate, pH 5/0.001% Triton X-100), a dye chamber **28** (containing a staining solution of 0.002% ethidium bromide in KH-phthalate buffer), and a binding/detection chamber **24**, which incorporates a binding surface which has been modified with a specific binding reagent comprising antibodies specific for bovine leukocyte cell surface antigens. The platform also comprises an overflow reservoir **23** and waste receptacle **25**. The milk sample is introduced, optionally after pretreatment, for example, to remove fat globules or other non-specific particulates, into the sample entry port on the platform. Platforms of the invention can be provided with wash buffer and stain or can be added to the platform immediately before use. The platform is rotated at a gradually increasing rate to move the excess fluid from the sample chamber into the overflow chamber. The speed is increased further to drive the sample into the binding/detection chamber, where it contacts the surface coated with the specific binding reagent. The milk is then incubated in the chamber for 30 minutes. Following incubation, a valve connecting the wash buffer reservoir to the binding/detection chamber is opened and fluid flow achieved, *e.g.*, by increasing the rotation rate or by actuating a thermal valve, so that the wash buffer flushes the milk sample out of the chamber and into the waste receptacle. After the wash buffer has replaced the milk in the binding/detection chamber, a valve connecting the dye chamber containing the staining solution to the binding/detection chamber is opened so that the staining solution fills the binding chamber and replaces the wash buffer. After allowing a sufficient time for the dye to stain the cells, the number of cells bound to the chamber are observed visually using source light at wavelengths between 510

On page 54, line 23 through page 55, line 3:

A platform arrangement useful in high throughput screening for insulin-stimulating drugs is illustrated in ~~Figure 2~~ Figures 2 and 2A. The platform consists of a cell accumulation chamber **24** in which cells are cultured; a test compound buffer chamber **29** for introducing a predetermined concentration of the test compound to the cell accumulation chamber; a dye chamber containing a cytological stain solution **27**; a wash buffer chamber **28**; and a waste receptacle chamber **25**.

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